

# The *Drosophila obscura* Species-group (Diptera, Drosophilidae) from Yunnan Province, Southern China

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**ABSTRACT**—Three new and two known species of the *Drosophila* (*Sophophora*) *obscura* species-group are reported from Yunnan Province, southern China. The *sinobscura* species-subgroup is newly established by *D. sinobscura*, *D. hubeiensis* and *D. luguensis* sp. nov. Geographic distribution of the *obscura* group in and around China is discussed, and a key to 10 Chinese species of the *obscura* group is provided.

**Key words:** Drosophilidae, *obscura* species-group, *sinobscura* species-subgroup, geographic distribution, China

## INTRODUCTION

The *Drosophila* (*Sophophora*) *obscura* species-group has been well studied in the field of evolutionary genetics since Dobzhansky and co-workers employed North American members of this group, *Drosophila pseudoobscura* Frolova, 1929, *D. miranda* Dobzhansky, 1935, and *D. persimilis* Dobzhansky & Epling, 1944, as materials for their intensive serial studies, "Genetics of Natural Populations", from Dobzhansky and Queal (1938) to Powell *et al.* (1976). This species-group presently includes 38 species and 3 subspecies. Most of them are distributed throughout the temperate zone of the Northern Hemisphere, with several species extending to tropical America (Throckmorton, 1975; Lakovaara and Saura, 1982) and a few species in the Afro-tropical region (Cariou *et al.*, 1988). The overall phylogenetic relationships within this group are still not well resolved, in spite of a number of previous studies on the phylogeny. Ambiguities still exist in some aspects such as: (1) Geographic origin of this group: Tropical African region or East Asian region? (2) History of the New World species: monophyletic or paraphyletic? (3) The phylogenetic positions of a

few Old World species, such as *D. subsilvestris* Hardy & Kaneshiro, 1968, *D. bifasciata* Pomini, 1940 and *D. tsukubaensis* Takamori & Okada, 1983, are ill-defined. Thus, more evidence, such as biogeographic information, would be indispensable for fully understanding the evolution of this group.

China had long been unexploited territory for the study of this group. Faunal surveys of drosophilid flies in the last decade uncovered the presence of the *obscura* group from China. Watabe *et al.* (1993) first reported 4 known species of this group from Xinjiang Uygur Autonomous Region, northwestern China. Thereafter, two new species of this group, *Drosophila sinobscura* Watabe, 1996 from Taiwan Island and *Drosophila hubeiensis* Sperlich & Watabe, 1997 from Hubei Province, central China, were discovered (Watabe *et al.*, 1996; Watabe and Sperlich, 1997).

Since 2000, we have engaged in a field survey in Yunnan Province, southern China, and found three new and two known species of the *obscura* group. In the present article, the new species are described with the establishment of a new species-subgroup, the *sinobscura* subgroup. A faunal comparison of the *obscura* group is made between southern China and several districts in the Palearctic Region, and a key to all 10 Chinese species of the *obscura* group is provided.

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## MATERIALS AND METHODS

**Observation:** External morphology was observed under a stereoscopic microscope, and metric characters were measured with an ocular micrometer. The male and female terminalia were detached from the body, treated with 10% KOH solution around 80°C for several min, and observed in a droplet of glycerol under a compound light microscope. Drawings were made with an ocular, mesh micrometer.

**Laboratory rearing:** Some morphological variations were detected between two allopatric populations of *D. hubeiensis*. Two representative iso-female strains were used for morphological comparisons: the Yunnan population (HY), collected from Gaoligongshan Nature Reserve, western Yunnan, and established in August 2000, and the Hubei population (HH), collected from Shennongjia Nature Reserve, Hubei, and established in July 1992. The adult flies were allowed to oviposit in a glass vial (30 mm in diameter, 105 mm in height) on usual *Drosophila* medium (10 ml) consisting of Baker's yeast-sucrose-cornmeal-agar (Watabe *et al.*, 1996). The rearing vials were maintained in incubator at 20±2°C under continuous light.

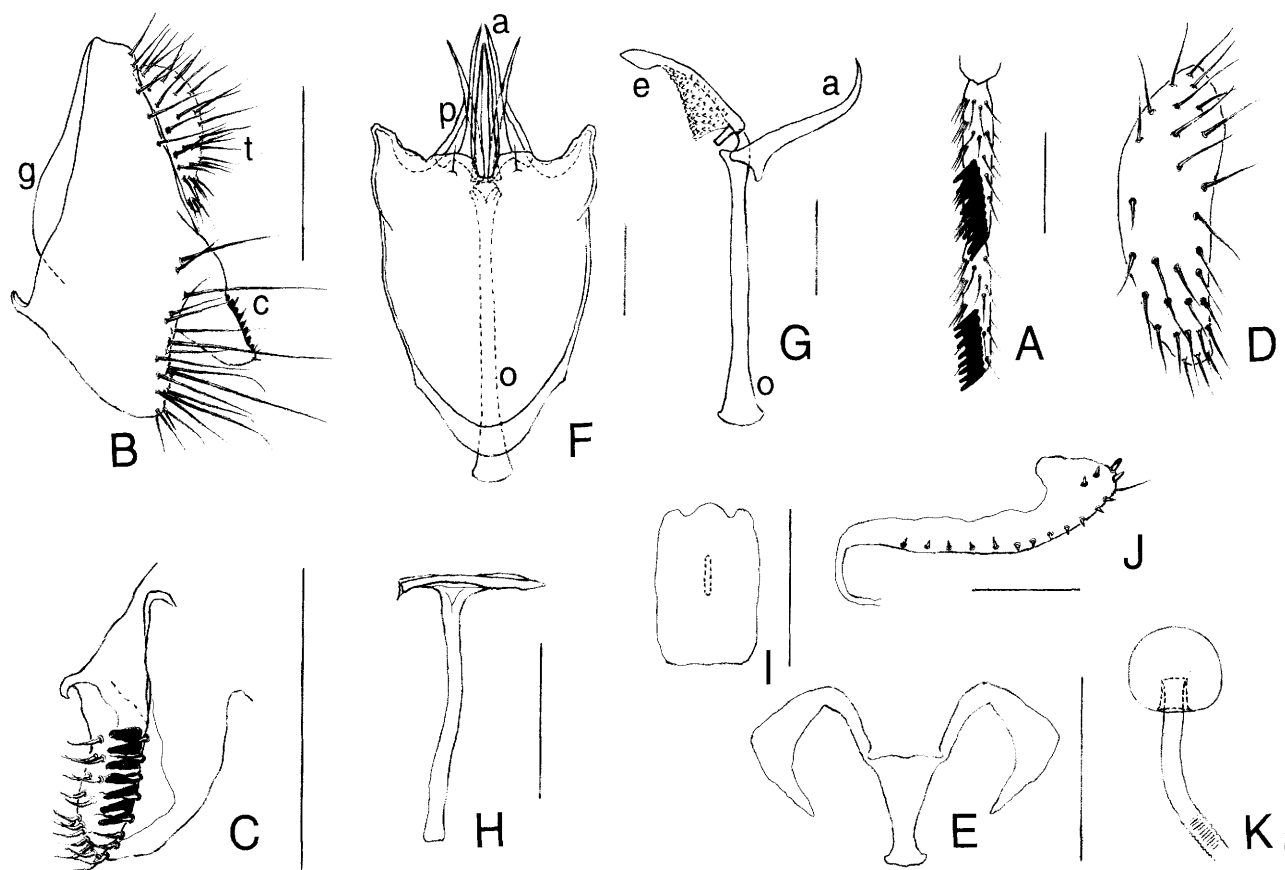
## RESULTS AND DISCUSSION

### Establishment of the *Drosophila sinobscura* species-subgroup

When Sturtevant (1942) originally established the

*obscura* group, he classified it into 2 species-subgroups, the *affinis* subgroup consisting of New World species and the *obscura* subgroup consisting of both New and Old World species. Since then, 3 more subgroups have been added: the Old World *subobscura*, the New World *pseudoobscura* and the Afro-tropical *microlabis* (Buzzati-Traverso and Scosiroli, 1955; Lakovara and Saura, 1982; O'Grady, 1999), leaving several species unassigned to any subgroup. Three species recently found from mainland China and Taiwan, *D. sinobscura*, *D. hubeiensis* and *D. luguensis* sp. nov., share a number of morphological characters and are all easily discriminated from the known subgroups by the diagnostic characters indicated below. Thus, we newly establish the *sinobscura* subgroup in the *obscura* group. In addition to morphological similarities, these three members constitute a compact cluster in the unrooted neighbor-joining dendrograms based on allozyme data and nucleotide sequences of the nuclear *Adh* gene and the mitochondrial *ND2* gene (Gao *et al.*, unpublished data)

**Diagnosis.** Scutum and scutellum black, without any longitudinal stripes. Acrostichal setulae in 8 rows. First tarsomeres of ♂ foreleg slightly longer than 2nd tarsomere, with large sex-comb present on both tarsomeres (Fig. 1A). Epandrium dark brown, with ventral portion paler. Median



**Fig. 1.** *Drosophila (Sophophora) luguensis* sp. nov. ♂ holotype from Yunnan, China; ♀ paratype, offspring from iso-female line collected in Lugufu, Yunnan. A, sex-combs on foreleg; B, male terminalia in lateral view; C, inner aspect of surstylus; D, cercus; E, decasternum; F, aedeagus and its adjacent structures in ventral view; G, aedeagus, paramere and gonopod in lateral view; H, ejaculatory apodeme in lateral view; I, ejaculatory apodeme in ventral view; J, oviscapit; K, spermatheca. Signs: a, paramere; c, surstylus; e, aedeagus; g, epandrium; o, aedeagal apodeme; p, gonopods; t, cercus. Scales = 0.1 mm.

piece of decasternum broadened, narrower than long, apically truncate or rounded. Aedeagus narrow, 1/3–1/4 of width of hypandrium; lateral plates sclerotized, apically with triangular expansion. Hypandrium pale brown, caudomedially slightly notched, with 1 pair of tiny paramedian setae on caudal margin. Spermatheca cone-shaped; introvert shallow, less than 1/2 height of outer capsule.

### Descriptions of *obscura* group species from Yunnan

Morphological characters commonly seen in all species examined (presence of dense interfacetal setulae on eye, coloration of pedicel, ocellar triangle and frons, chaetotaxy of postpronotal lobe, etc.) are referred to in the description of the first species but not repeated for the other species.

### *Drosophila (Sophophora) luguensis* Gao & Toda sp. nov.

(Fig. 1A–K)

♂, ♀. Head: Eye red, with thick interfacetal setulae. Ocellar triangle black. Pedicel dark brown, with a few setae; 1st flagellomere grayish brown. Frons black, 0.41 in holotype (range in 19 paratypes: 0.41–0.55) as broad as head, with several interfrontal setulae. Arista with 3 dorsal and 2 ventral branches besides terminal bifurcation. Anterior reclinate orbital seta (Orb2) 0.45 (0.32–0.50) length of posterior reclinate orbital seta (Orb1); proclinate orbital seta (Orb3) 0.83 (0.64–0.92) length of Orb1. Face black; facial carina dark brown, moderate. Clypeus black. Gena brown, 0.29 (0.27–0.31) as broad as maximum diameter of eye. Subvibrissal seta (Or2) 0.45 (0.39–0.56) length of vibrissa (Or1). Palpus yellow, with 1 prominent apical seta and several ventral ones.

Thorax: Scutum and scutellum black, without longitudinal stripes. Postpronotal lobe dark brown, with 2 prominent setae (Hu). Anterior dorsocentral seta (DcA) 0.45 (0.45–0.76) length of posterior dorsocentral one (DcP); length distance of dorsocentral setae 0.47 (0.33–0.56) cross distance. Acrostichal setulae (Ac) in 8 rows. Anterior/posterior katapisternal seta length (Sterno-index) 0.57 (0.47–0.61).

Wing hyaline, slightly clouded along costa in ♂. Veins brown;  $R_{2+3}$  gently curved to costa at tip;  $R_{4+5}$  and  $M_1$  nearly parallel. Wing indices:  $C=2.93$  (2.63–3.28);  $4V=1.98$  (1.70–2.50);  $4C=0.88$  (0.83–1.05);  $5x=1.91$  (1.31–2.01);  $Ac=2.55$  (2.23–2.68);  $C3F=0.33$  (0.31–0.43).  $C_1$  setae 2, less-differentiated. Halteres white.

Legs yellowish brown. Foreleg 1st tarsomere slightly longer than 2nd tarsomere. Sex-comb teeth 10 (9–11) on 1st tarsomere, 8 (8–10) on 2nd tarsomere (Fig. 1A).

Abdomen: Tergites uniformly brownish black; sternites gray in ♂, pale gray in ♀.

Male terminalia (Fig. 1B–I): Epandrium dark brown, paler on ventral portion, lacking pubescence, with 5 (range in 9 ♂ paratypes: 4–6) setae along posterior margin of upper half and 19 (15–21) setae on caudo-ventral margin of lower half. Surstylus pale brown, with 8 (7–9) peg-like prenisetae and ca. 15 trichoid setae. Cercus oval, brown, glabrous, with 30 (27–35) setae distributed thickly ventrally and sparsely

dorsally. Decasternum less-sclerotized; median lobe rectangular; lateral flap large. Aedeagus yellowish brown, narrow (ca. 1/4 of hypandrial width), articulated with apodeme; lateral plates sclerotized, apically with triangular expansion, basally with quadrate knob in lateral view; median membrane covered with numerous wart-like serrations; apodeme dark brown, 2.28 (2.03–2.31) times as long as aedeagus. Paramere longer than aedeagus, sword-shaped in lateral view, apically pointed, with 6 minute sensilla arranged longitudinally on basal to subapical, lateral surface. Gonopods as long as aedeagus, apically fused to aedeagal, basal process. Hypandrium pale brown, caudo-medially slightly notched, with 1 pair of tiny paramedian setae on caudal margin. Ejaculatory apodeme plate pale brown, quadrate; stalk dark brown, longer than apical plate.

Female terminalia (Fig. 1J–K): Oviscapt yellowish brown, with 2–3 lateral and 11–17 marginal peg-like ovisensilla and subterminal trichoid seta; antero-ventral bridge long. Spermatheca dark brown, cone-shaped; introvert ca. 2/5 height of outer capsule.

Measurements: Body length = 2.33 mm (range in 9 ♂ paratypes: 1.92–2.56, range in 10 ♀ paratypes: 2.93–3.39). Thorax length including scutellum = 1.03 mm (♂: 0.81–1.11; ♀: 1.16–1.29). Wing length = 2.56 mm (♂: 2.10–2.67; ♀: 2.65–2.97), and its width = 1.10 mm (♂: 0.92–1.19; ♀: 1.14–1.32).

Holotype, ♂, China: Lugu Lake Nature Reserve, (ca. 2800–3000 m above sea level), Lijiang, Yunnan Province, 26.vii.2001, ex banana traps (Gao and Watabe leg.); deposited in Kunming Institute of Zoology, Chinese Academy of Sciences, Yunnan, China (KIZ).

Paratypes, China: 9 ♂, 25.vii.2001; 20 ♂, 26.vii.2001; 15 ♂, 27.vii.2001, same collection site as holotype; 16 ♂, 15 ♀, offspring from iso-female line, collected in the type locality and established on 25–26.vii.2001; 50 ♂, 5 ♀ deposited in KIZ, and 10 ♂, 10 ♀ in Systematic Entomology, the Hokkaido University Museum, Hokkaido University, Sapporo, Japan (SEHU).

*Distribution.* China: Yunnan.

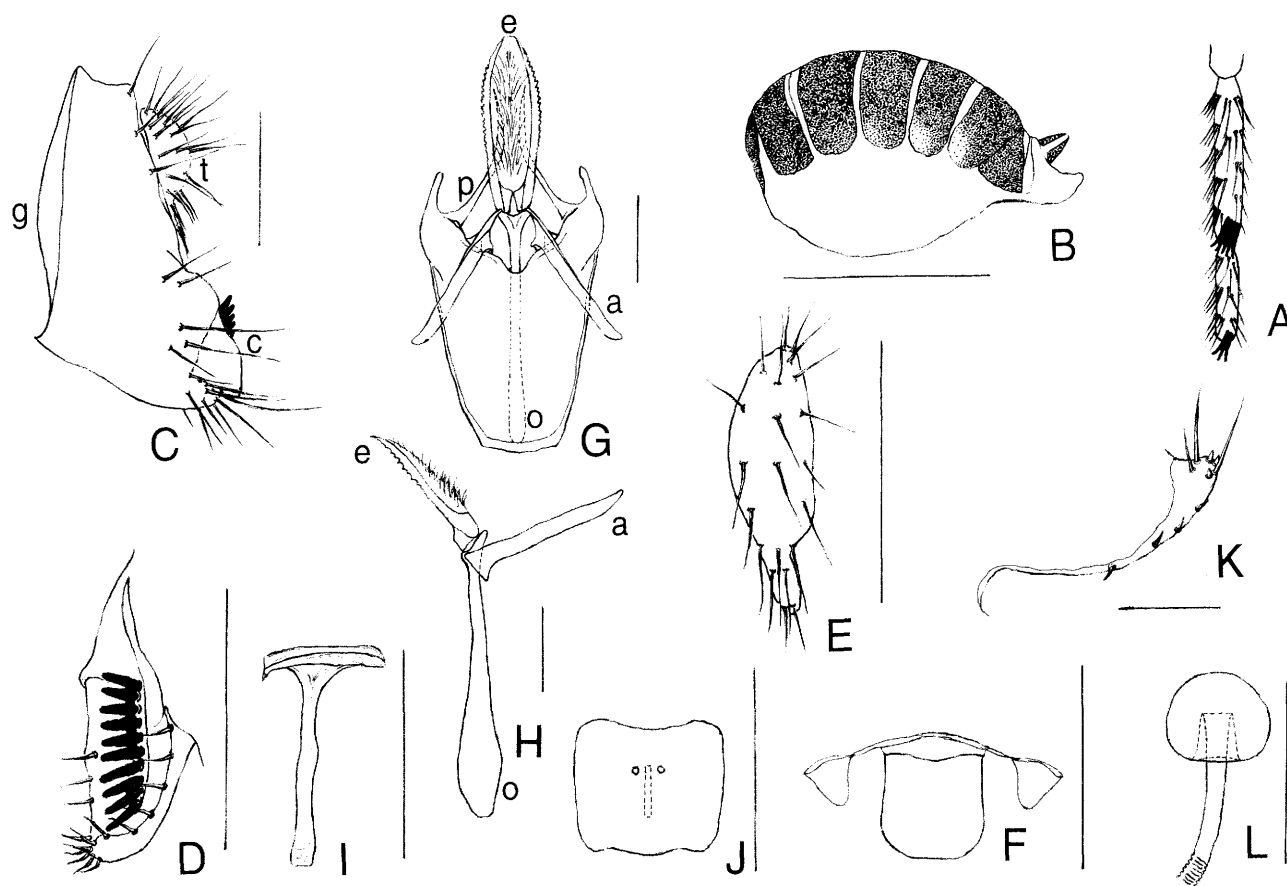
*Relationships and diagnosis.* *Drosophila luguensis* can be clearly distinguished from *D. sinobscura* and *D. hubeiensis* by the aedeagus with serrations on median membrane and with the quadrate knob at base (in both *D. sinobscura* and *D. hubeiensis*, the aedeagus is smooth on the median membrane and the basal knob is triangular). Further, the phallosomal index (the relative length of aedeagal apodeme to the aedeagus) of *D. luguensis* (2.03–2.31) is larger than those of *D. sinobscura* (1.51–1.84) and *D. hubeiensis* (1.49–1.67).

*Etymology.* Pertaining to the type locality.

### *Drosophila (Sophophora) dianensis* Gao & Watabe sp. nov.

(Fig. 2A–L)

♂, ♀. Head: Pedicel dark yellow; 1st flagellomere grayish yellow. Frons 0.48 in holotype (range in 7 paratypes:



**Fig. 2.** *Drosophila (Sophophora) dianensis* sp. nov. ♂ holotype, ♀ paratypes, from Yunnan, China. A, sex-combs on foreleg; B, female abdominal tergites in lateral view; C, male terminalia in lateral view; D, inner aspect of surstylus; E, cercus; F, decasternum; G, aedeagus and its adjacent structures in ventral view; H, aedeagus, paramere and gonopod in lateral view; I, ejaculatory apodeme in lateral view; J, ejaculatory apodeme in ventral view; K, oviscapt; L, spermatheca. Scales = 0.1 mm, except for Fig. 2B (1.0 mm). Signs as in Fig. 1.

0.46–0.53) as broad as head. Arista with 3 dorsal and 2 ventral branches. Orb2 0.45 (0.30–0.49) length of Orb1; Orb3 0.74 (0.64–0.77) length of Orb1. Face black; facial carina broadened, dark yellow. Gena 0.30 (0.26–0.35) as broad as maximum diameter of eye. Or2 weak, 0.60 (0.45–0.64) length of Or1. Palpus yellow, with 1 prominent apical seta and 1 lateral one.

Thorax: Scutum black, without longitudinal stripes; scutellum dark brown, marginally black. DcA 0.67 (0.61–0.70) length of DcP; length distance of dorsocentral setae 0.41 (0.38–0.52) cross distance. Ac in 8 regular rows. Sterno-index 0.51 (0.51–0.60).

Wing pale yellow. Indices: C=2.88 (2.32–3.00); 4V=1.93 (1.88–2.50); 4C=0.93 (0.88–1.29); 5x=2.12 (1.80–2.22); Ac=2.47 (2.31–3.05), C3F=0.34 (0.34–0.49).

Legs dark brown. Foreleg 1st tarsomere much longer than 2nd tarsomere. Sex-combs strongly oblique against main axis of tarsomeres, with 4 (4–6) teeth on 1st tarsomere and 3 (3–5) teeth on 2nd tarsomere (Fig. 2A).

Abdomen: Tergites brownish black, with small faint pale spot on antero-ventral corner of 4T and distinct pale spots on antero-ventral corner of 5–6Ts in ♀ (Fig. 2B). Sternites pale gray, with 13 (11–15) fine setae on ♂ 5S.

Male terminalia (Fig. 2C–J): Epandrium dark brown, ventrally paler, lacking pubescence, with 3 (range in 3

paratypes: 4–6) setae along posterior margin and 14 (15–19) setae on caudo-ventral portion. Surstylus dark brown, with 10 (9–10) peg-like prensisetae and 16 (14–16) trichoid setae. Cercus grayish brown, broadened on upper half and gently tapering downward, with 21 (21–23) setae. Decasternum sclerotized; median lobe broadened, nearly quadrate. Aedeagus ca. 1/3 as broad as hypandrium, articulated with apodeme; lateral plate much-sclerotized, with numerous serrations subbasally to subapically and fine setulae antero-ventrally; median membrane covered with fine, long (proximally) or short (distally) setulae; apodeme brown, longer than aedeagus. Paramere yellowish brown, as long as aedeagus, sword-shaped in lateral view, apically pointed, with 12–13 min sensilla arranged longitudinally on basal to subapical, lateral surface. Gonopods separated from each other, shorter than aedeagus; aedeagal basal process almost degenerated. Hypandrium pale brown, with 1 pair of tiny paramedian setae on deeply concaved, caudal margin. Ejaculatory apodeme plate brown, gently convex on cephalic margin, with 1 pair of small pits medially; stalk dark brown, longer than apical plate.

Female terminalia (Fig. 2K–L): Oviscapt grayish yellow, with 2–3 lateral and 6–10 marginal ovisensilla; dorsoterminal one trichoid, ca. 2/3 length of ventro-subterminal seta which being as long as maximum width of oviscapt; antero-

ventral bridge long. Spermatheca brown, cone-shaped; introvert ca. 3/5 height of outer capsule.

Measurements: Body length = 2.25 mm (3 ♂ paratypes: 2.01–2.10; 4 ♀ paratypes: 2.21–2.75). Thorax length including scutellum = 0.92 mm (♂ : 0.92–1.01; ♀ : 0.84–1.10). Wing length = 1.98 mm (♂ : 2.22–2.32; ♀ : 1.92–2.38), and its width = 0.88 mm (♂ : 1.01–1.10; ♀ : 0.92–1.14).

Holotype, ♂, China: Jiao-Ye Park (ca. 1950 m a.s.l.), Kunming, Yunnan, 27.iv.2002, ex banana traps (Gao leg.); deposited in KIZ.

Paratypes, China: 1 ♀, 26.ii.2001; 1 ♀, 1 ♂, 3.iii.2001; 1 ♂, 6.iii.2001; 1 ♂, 13.iii.2001; 1 ♀, 8.iii.2001; 1 ♀, 27.iv.2002, the same collection site as holotype; 2 ♂, 2 ♀ deposited in KIZ, and the remaining in SEHU.

*Distribution.* China: Yunnan.

*Relationships and diagnosis.* *Drosophila dianensis* closely resembles *D. subsilvestris*, which is distributed from Europe to Central Asia, in having the sex-combs with small numbers of teeth, the broadened aedeagus with much-sclerotized lateral plates, the rudimentary basal process of aedeagus and the 2 long trichoid setae at the apex of oviscapt, but can be clearly distinguished from it by the aedeagal lateral plates with serrations in ♂ and by the

absence of pale spots on lateral margins of the 2nd abdominal tergite in ♀ (in *D. subsilvestris*, the aedeagal lateral plates are smooth and the ♀ 2nd tergite has pale spots on its lateral margins).

*Etymology.* Pertaining to “Dian”, the name of an ancient nation having occupied the area surrounding the type locality.

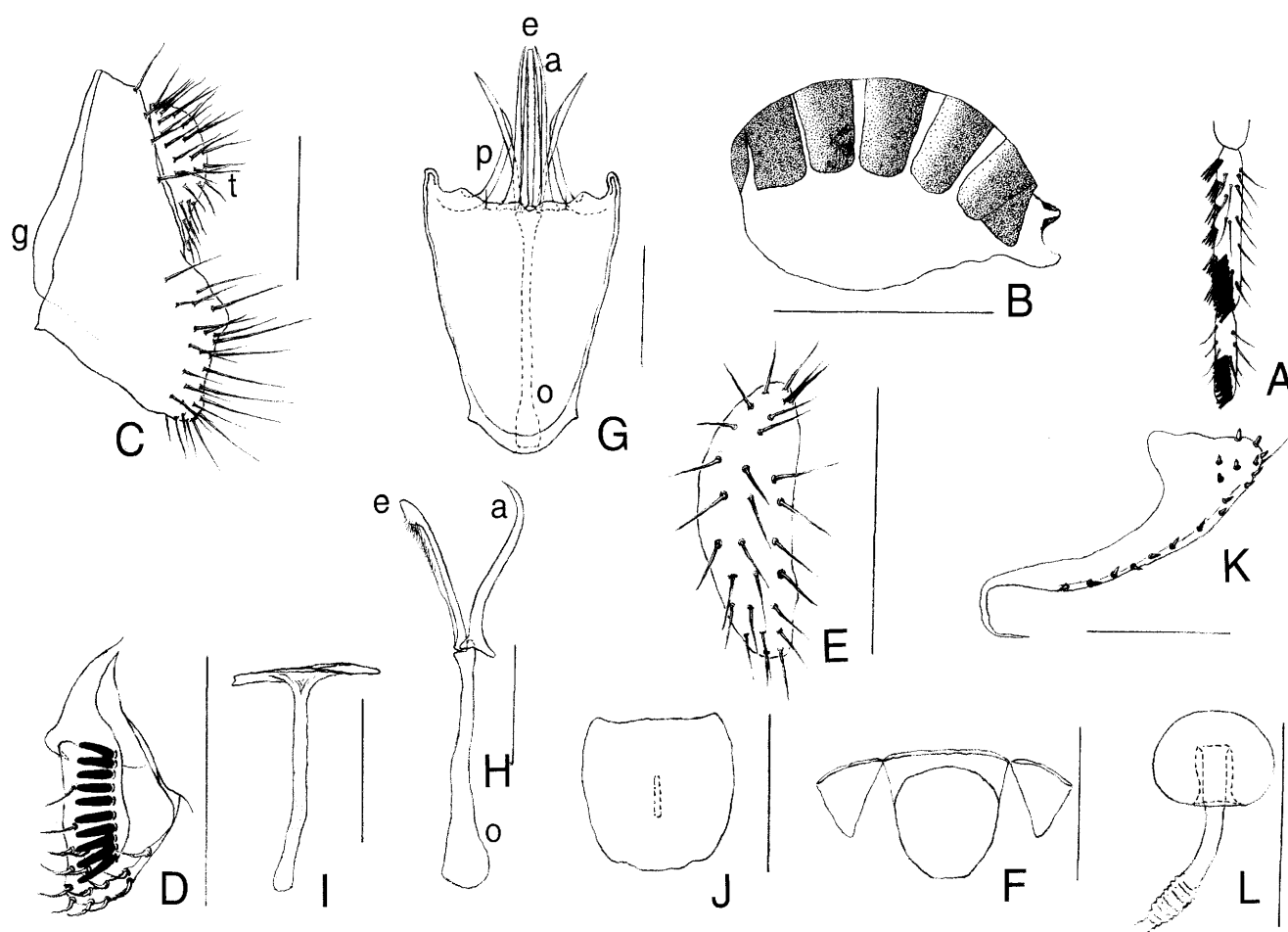
***Drosophila (Sophophora) limingi* Gao & Watabe sp. nov.**

(Fig. 3A–L)

♂, ♀. Head: First flagellomere of pedicel grayish brown. Frons 0.45 in holotype (range in 7 paratypes: 0.43–0.54) as broad as head. Arista with 3 (3–4) dorsal and 2 (2–3) ventral branches. Orb2 0.42 (0.38–0.48) length of Orb 1; Orb3 0.68 (0.61–0.76) length of Orb1. Gena yellowish brown, 0.40 (0.30–0.47) as broad as maximum diameter of eye. Or2 0.50 (0.32–0.54) length of Or1. Palpus with 1 prominent apical seta and several ventral ones.

Thorax: Scutum and scutellum black, without longitudinal stripes. DcA 0.65 (0.65–0.71) length of DcP; length distance of dorsocentral setae 0.62 (0.36–0.62) of cross distance. Ac in 8 rows. Sterno-index 0.51 (0.47–0.55).

Wing hyaline, slightly clouded. Veins brown. Indices:



**Fig. 3.** *Drosophila (Sophophora) limingi* sp. nov. ♂ holotype, ♀ paratypes, from Yunnan, China. A, sex-combs on foreleg; B, female abdominal tergites in lateral view; C, male terminalia in lateral view; D, inner aspect of surstylus; E, cercus; F, decasternum; G, aedeagus and its adjacent structures in ventral view; H, aedeagus, paramere and gonopod in lateral view; I, ejaculatory apodeme in ventral view; J, ejaculatory apodeme; K, oviscapt; L, spermatheca. Scales = 0.1 mm, except for Fig. 3B (1.0 mm). Signs as in Fig. 1.

C=2.70 (2.30–3.20); 4V=2.49 (1.82–2.33); 4C=1.26 (0.91–1.30); 5x=1.76 (1.68–2.33); Ac=2.40 (2.20–2.70), C3F=0.33 (0.31–0.42). Halteres pale yellow.

Legs dark brown; tibiae and tarsi paler. Foreleg 1st tarsomere ca. 1.8 times as long as 2nd tarsomere. Sex-comb teeth 10 (8–11) on 1st tarsomere and 10 (8–11) on 2nd tarsomere (Fig. 3A).

Abdomen: Tergites brownish black, anteriorly narrowly pale yellow on 4–6Ts in ♀ (Fig. 3B). Sternites dark gray in ♂, pale gray in ♀.

Male terminalia (Fig. 3C–J): Epandrium yellowish brown, glabrous, with 4 (range in 4 ♂ paratypes: 3–7) setae along posterior margin of upper half and 27 (22–29) setae on ventral margin of lower half. Surstylus brown, with 10 (9–10) peg-like prensisetæ and 15 (15–21) trichoid setae, and hook-shaped projection strongly curved inward at dorso-caudal corner. Cercus oval, ventrally darker, with 28 (24–30) setae nearly evenly distributed. Decasternum comprising broadened median lobe (dark area somewhat polygonal) and flap-bearing lateral arms. Aedeagus yellowish brown, narrow (ca. 1/4 of hypandrial width), articulated with apodeme, with fine setulae; caudo-ventrally; lateral plates sclerotized, apically expanded triangularly in lateral view; median membrane distally hirsute; apodeme brown, longer than aedeagus. Paramere as long as aedeagus, sword-shaped in lateral view, apically pointed, with 6–7 min sensilla arranged longitudinally from basal to submedial portion on lateral surface. Gonopods separated from each other, slightly shorter than aedeagus, each apically fused to aedeagal basal process. Hypandrium pale brown, caudo-medially slightly notched, with 1 pair of small paramedian setae on caudal margin. Ejaculatory apodeme plate light brown, quadrate, with 1 pair of small pits medially; stalk dark brown, longer than apical plate.

Female terminalia (Fig. 3K–L): Oviscapt grayish yellow, with 3–4 lateral and 13–16 marginal peg-like ovisensilla, and 1 subterminal trichoid seta; antero-ventral bridge long. Spermatheca pale brown, cone-shaped; introvert ca. 3/5 height of outer capsule.

Measurements: Body length = 2.01 mm (range in 4 ♂ paratypes: 1.92–2.15, range in 3 ♀ paratypes: 2.21–2.38). Thorax length including scutellum = 0.81 mm (♂: 0.79–0.89, ♀: 0.80–0.92). Wing length = 1.88 mm (♂: 1.72–1.99, ♀: 1.88–2.22), and its width = 0.84 mm (♂: 0.79–0.97, ♀: 0.88–1.02).

Holotype, ♂, China: Jiao-Ye Park (ca. 1950 m a.s.l.), Kunming, Yunnan Province, 26.iii.2001, ex banana trap (Gao and Watabe leg.); deposited in KIZ.

Paratypes, China: 2 ♂, 1 ♀, 8.iii.2001; 2 ♂, 13.iii.2001; 1 ♀, 15.iii.2001; 1 ♀, 23.iii.2001; 3 ♂, 26.iii.2001; 1 ♂, 27.iv.2002, same collection site as holotype; 5 ♂, 2 ♀ deposited in KIZ, and the remaining in SEHU.

*Distribution.* China: Yunnan.

*Relationships and diagnosis.* *Drosophila limingi* is somewhat similar to *D. tsukubaensis* in having relatively small numbers of teeth on the sex-combs and the remark-

able hook-shaped projection at the dorso-caudal corner of surstylus, and to *D. obscura* Fallén, 1823 in the general morphology of decasternum, but *D. limingi* can be distinguished from *D. tsukubaensis* by the broadened median lobe of decasternum (in *D. tsukubaensis* it is narrow), and from *D. obscura* by the large projection at the dorso-caudal corner of surstylus (in *D. obscura* it is rudimentary), in addition to the setae being distributed evenly on the cercus.

*Etymology.* Patronym, in honor of the late Professor Liming Shi (KIZ), who initiated the joint research project on Evolutionary Genetics and Phylogeny of Chinese Drosophilidae between China and Japan.

### *Drosophila (Sophophora) tsukubaensis* Takamori & Okada

*Drosophila (Sophophora) tsukubaensis* Takamori & Okada, 1983: 265.

*Specimen examined.* 1 ♂, 13. iii. 2001; 1 ♂, 8. iv. 2002; 1 ♂, 29. iv. 2002; Jiao-Ye Park (ca. 1950 m a.s.l.), Kunming, Yunnan, ex banana traps.

*Distribution.* Japan; China: Yunnan.

*Remarks.* In Japan, *Drosophila tsukubaensis* was known from Tokyo and its adjacent prefectures, and it occurs in early spring in Yunnan and Japan (Takamori and Okada, 1983; Okada, 1988).

### *Drosophila (Sophophora) hubeiensis* Sperlich & Watabe

*Drosophila (Sophophora) hubeiensis* Sperlich & Watabe, in Watabe & Sperlich, 1997: 622.

*Specimens examined.* 18 ♂, 13 ♀; 11. viii. 2000; from timber piles; 12 ♂, 14 ♀; 15. viii. 2000, ex banana traps in streamsides, Gaoligongshan National Nature Reserve, Yunnan, China, ca. 2300 m a.s.l. (Watabe leg.).

*Distribution.* China: Hubei, Sichuan, Yunnan (n. loc.).

*Geographic variation.* Some morphological, but slight, differences are found between the Yunnan population (HY) and the type-locality population of Hubei (HH) in *D. hubeiensis*. In HY the legs are generally pale brown with dark areas on the lower portion of femur and on the upper portion of tibia and the spermatheca is dark brown, whereas in HH the legs are uniformly dark brown and the spermatheca pale brown. It is well known in many drosophilid species that the body color is more or less changeable owing to rearing temperatures (Watabe, 1977), but the above difference between HY and HH is relatively stable.

Table 1 shows intra-specific variations in 31 quantitative characters between the two geographic populations of *D. hubeiensis*, HY and HH, reared under the same laboratory conditions described in "Materials and Methods". As for the characters Nos. 1 to 4 and 8, the comparison was made for each sex separately. The intra-specific differences proved statistically significant ( $p < 0.05$ ) in 19 (Nos. 1, 3, 4 ♀, 8, 9, 10, 12, 17, 20, 22, 23 and 27–30) out of 36 comparisons, although the ranges overlapped between the two geographic populations in all characters, excepting for No. 1 (BL in ♀) and No. 3 (WL). Further, HY is much easier to be

**Table 1.** Comparison of 31 quantitative characters between two geographic strains of *D. hubeiensis*, HY and HH.

Quantitative characters		HY*			HH**			Difference between HY and HH (t-test)
		Mean±S.D.	Range	n	Mean±S.D.	Range	n	
1. Body length (mm)	♂	2.17±0.13	1.88–2.48	20	2.47±0.16	2.28–2.76	20	p<0.01
	♀	2.35±0.12	2.08–2.56	20	3.08±0.24	2.84–3.44	20	p<0.01
2. Thorax length (mm)	♂	1.01±0.08	0.82–1.11	10	1.04±0.07	0.92–1.16	10	ns
	♀	1.22±0.11	1.13–1.54	10	1.24±0.07	1.12–1.32	10	ns
3. Wing length (mm)	♂	2.22±0.07	2.11–2.32	10	2.72±0.11	2.56–2.88	10	p<0.01
	♀	2.44±0.07	2.32–2.57	10	3.20±0.10	3.08–3.40	10	p<0.01
4. Wing width (mm)	♂	0.99±0.04	0.94–1.06	10	1.04±0.07	0.92–1.16	10	ns
	♀	1.12±0.05	1.08–1.19	10	1.24±0.05	1.12–1.28	10	p<0.01
5. Frons width / head width		0.47±0.02	0.44–0.52	20	0.42±0.03	0.37–0.49	20	p<0.01
Arista								
6. No. of upper branches		3±0.00	3– 3	40	3.00±0.31	2– 4	42	ns
7. No. of lower branches		2±0.00	2– 2	40	1.83±0.38	1– 2	42	ns
8. Tiny setulae on palpus	♂	11.73±2.58	9–17	30	13.60±0.34	9–18	30	p<0.01
	♀	13.60±1.54	10–16	30	15.87±1.74	12–19	30	p<0.01
9. Gena width / eye diameter		0.26±0.03	0.22–0.31	20	0.19±0.03	0.14–0.26	20	p<0.01
10. Or2/Or1		0.45±0.06	0.36–0.58	20	0.39±0.09	0.25–0.67	20	p<0.05
11. Orb2/Orb1		0.43±0.06	0.32–0.53	20	0.46±0.08	0.33–0.64	20	ns
12. Orb3/Orb1		0.74±0.08	0.56–0.87	20	0.81±0.13	0.55–1.13	20	p<0.05
Thorax								
13. Lower / upper Hu length		0.97±0.09	0.83–1.18	20	0.99±0.11	0.76–1.17	20	ns
14. DcA / DcP length		0.69±0.04	0.60–0.76	20	0.71±0.08	0.55–0.88	20	ns
15. Length distance / cross distance of Dc		0.45±0.04	0.38–0.53	20	0.44±0.06	0.33–0.54	20	ns
16. SctB / SctA length		0.96±0.05	0.84–1.07	20	0.94±0.08	0.83–1.13	20	ns
17. Sterno-index		0.55±0.08	0.41–0.68	19	0.68±0.09	0.54–0.80	20	p<0.01
18. Distance from SctA to SctB/ distance between SctAs		1.12±0.10	0.89–1.22	20	1.12±0.14	0.86–1.37	20	ns
Wing indices								
19. C		2.75±0.16	2.45–3.00	20	2.79±0.23	2.21–3.21	20	ns
20. 4V		1.95±0.13	1.85–2.26	20	1.82±0.18	1.39–2.25	20	p<0.05
21. 4C		0.95±0.07	0.74–1.07	20	0.94±0.10	0.80–1.19	20	ns
22. 5X		1.83±0.21	1.44–2.15	20	1.56±0.19	1.14–1.83	20	p<0.01
23. Ac		2.17±0.09	2.00–2.32	20	2.33±0.25	2.00–2.80	20	p<0.05
24. C3F		0.40±0.05	0.33–0.48	20	0.40±0.05	0.32–0.50	20	ns
Sex-comb								
25. No. of teeth on 1st tarsomere		10.23±0.84	9–12	30	10.53±1.07	8–14	30	ns
26. No. of teeth on 2nd tarsomere		9.50±0.81	8–11	30	9.37±1.07	7–11	30	ns
27. No. of setae on ♂ 5S		16.45±1.99	14–21	20	14.45±1.46	11–16	20	p<0.01
Epandrium								
28. No. of setae on upper half		5.40±0.40	5– 6	20	6.10±0.91	4– 7	20	p<0.01
29. No. of setae on lower half		16.95±1.40	15–19	20	14.85±1.35	12–17	20	p<0.01
Oviscapt								
30. No. of lateral ovisensilla		2.33±0.47	2– 3	30	2.73±0.58	2– 4	30	p<0.01
31. No. of marginal ovisensilla		13.8±1.05	12–16	30	13.47±1.01	12–15	30	ns

\*HY, Yunnan strain; HH, Hubei strain. \*\*Watabe and Sperlich (1997)

reared in the laboratory than HH.

*Drosophila hubeiensis* is distributed from middle to southern China, but is strongly restricted to high mountains at 2000–3000 m a.s.l. It is reasonable to assume that the gene exchange between local populations is severely limited by surrounding lowlands with hot and dry climate. We have detected some differences in cross-ability between geographic strains of *D. hubeiensis* and its closely related species and will publish the results elsewhere.

### Faunal comparison

Including the 3 new species described in the present study, a total of 10 *obscura* group species have been recorded from China, and Yunnan is the southern-most location within the distribution range of the *obscura* group in the Eurasian Continent. The Chinese members of the *obscura* group are largely divided into two groups from a chorological point of view. Most species found in northern China show Palearctic distribution patterns. Both *D. alpina* Burla, 1948 and *D. bifasciata* are widely distributed from Europe to Japan throughout northern China. *Drosophila subsilvestris* and *D. subobscura* Collin, 1936 are western Palearctic species, and the eastern border of their distribution lies in the Ili and the Altay Mountains in Xinjiang (Watabe *et al.*, 1993). On the other hand, the species discovered from southern China, *D. hubeiensis*, *D. luguensis*, *D. limingi* and *D. dianensis*, are endemic to the Oriental Region.

The *obscura* group species composition of Yunnan (5 spp.) was compared with those of mainland Europe (9 spp.), Central Asia (5 spp.) covering Uzbekistan, Kazakhstan and northwestern China, Northeast Asia (4 spp.) covering north-eastern China, Korea and Far East of Russia, and the Japan Islands (5 spp.; *Drosophila eniwa* Takada, Beppu & Toda, 1979 was excluded from the present analysis, because of its questionable status), based on the following data sources (Bächli and Rocha-Pite, 1981; Wheeler, 1981; Lakovaara and Saura, 1982; Watabe *et al.*, 1993; Watabe and

Sidorenko, 1997, etc.). Faunal similarity between two districts was evaluated by Jaccard's coefficient of similarity (Udvardy, 1969):

$$S=c/(a+b-c),$$

where *c* is the number of species common to both districts and *a* or *b* is the number of species occurring in either district. The similarity matrix resulting from the ten pair-wise calculations was then subject to a cluster analysis. Fig. 4 clearly shows that Yunnan is quite isolated from other districts of the Palearctic Region in the species composition of the *obscura* group.

Huge arid zones of the Gobi and the Taklamakan in Central Asia may disturb the range expansion of *obscura* group species between Asia and Europe. In China, the Central Plain spreads along middle and lower reaches of the River Yangtze, and it may also disturb the faunal exchange in montane drosophilids between southern and northern China. Since the *obscura* group is undoubtedly a temperate forest element of drosophilid fauna in the Northern Hemisphere, it is likely that those geographic barriers have restricted their range expansion and have reinforced genetic differentiation leading to speciation. The present faunal study clearly demonstrates that southern China is an important place where an adaptive radiation of the *obscura* group might have occurred.

### Check-list of *obscura* group species in China

The *obscura* species-subgroup

- 1) *Drosophila (Sophophora) alpina* Burla, 1948  
China (Xinjiang); Europe, Russia, Mongolia, Korea, Japan
- 2) *D. (S.) bifasciata* Pomini, 1940  
China (Heilongjiang, Jilin, Xinjiang, Jiangsu\*, Sichuan\*, Zhejiang\*, Yunnan\*, Taiwan\*); Europe, Russia, Turkmenia, Kazakhstan, Uzbekistan, Korea, Japan, India\* (Some previous records with asterisks should be re-examined in the light of the present knowledge.)

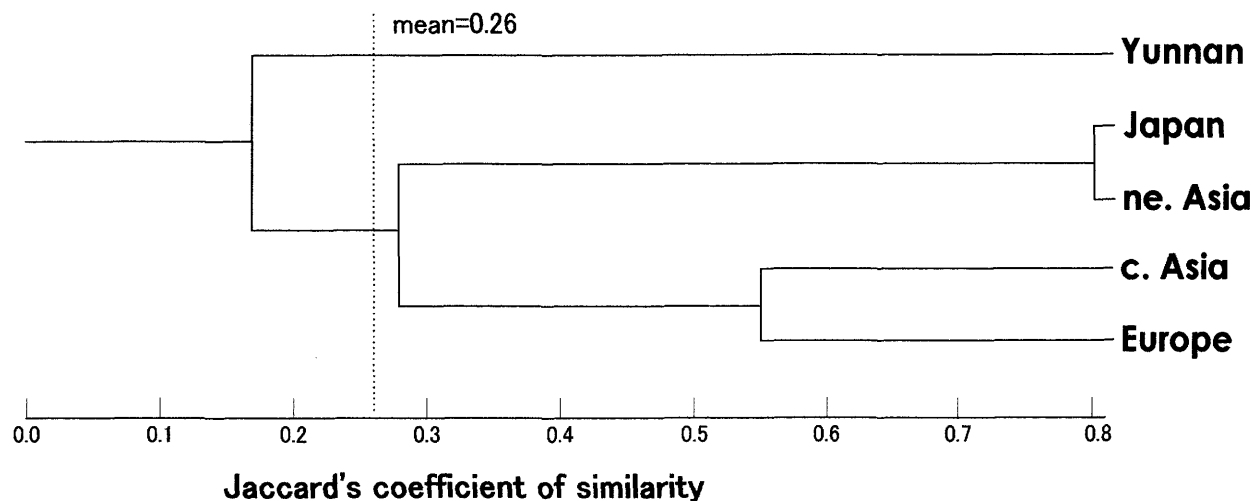


Fig. 4. Comparison of the *obscura*-group species composition based on Jaccard's coefficient of similarity among five districts in Eurasia. c, central; ne, northeastern.



- 3) *D. (S.) dianensis* sp. nov.  
China (Yunnan)
  - 4) *D. (S.) subsilvestris* Hardy & Kaneshiro, 1968  
China (Xinjiang); Europe, Russia
- The *sinobscura* species-subgroup
- 5) *D. (S.) hubeiensis* Sperlich & Watabe, 1997  
China (Hubei, Sichuan, Yunnan)
  - 6) *D. (S.) luguensis* sp. nov.  
China (Yunnan)
  - 7) *D. (S.) sinobscura* Watabe, 1996  
China (Taiwan)
- The *subobscura* species-subgroup
- 8) *D. (S.) subobscura* Collin, 1936  
China (Xinjiang); Azores Is., Madeira Is., Canary Is., northern Africa, Europe, Russia, Near East, Turkmenia, Kazakhstan, Uzbekistan, Chile
- Ungrouped
- 9) *D. (S.) limingi* sp. nov.  
China (Yunnan)
  - 10) *D. (S.) tsukubaensis* Takamori & Okada, 1983  
China (Yunnan); Japan

# Key to the species

0. Blackish species with sex-combs on ♂ 1st and 2nd tarsomeres; palpus with 1 prominent seta at tip; testis not or only slightly coiled; ventral receptacle usually short; female oviscapt usually white (*Drosophila (Sophophora) obscura* species-group)..... 1
1. First tarsomere of ♂ foreleg nearly as long as 2nd tarsomere ..... 2  
—First tarsomere of ♂ foreleg longer than 1.5 times of 2nd tarsomere ..... 6
2. Abdominal, anterior tergites pale; sex-combs occupying entire lengths of 1st and 2nd tarsomeres; surstylus without teeth-like prenisetae..... *alpina* Burla  
—Anterior, abdominal tergites dark; sex-combs not occupying entire lengths of 1st and 2nd tarsomeres; surstylus with teeth-like prenisetae ..... 3
3. C3F>0.5.....*subobscura* Collin  
—C3F≤0.5 ..... 4
4. Aedeagal, median membrane covered with numerous serrations; aedeagal apodeme longer than 2 times of aedeagus..... *luguensis* sp. nov.  
—Aedeagal, median membrane without serrations; aedeagal apodeme shorter than 2 times of aedeagus..... 5
5. Legs dark brown.....*hubeiensis* Sperlich & Watabe  
—Legs brown..... *sinobscura* Watabe
6. Sex-combs strongly oblique against main axis of tarsomeres, with side teeth diverged outwards and less than 5 teeth in distal sex-comb; female with distinct pale spots on abdominal tergites and 2 long, trichoid setae at apex of oviscapt..... 7  
—Sex-combs slightly oblique against main axis of tarsomeres, with side teeth not or only slightly diverged outwards and more than 6 teeth in distal sex-comb; female without pale spots on abdominal tergites and with only 1

- long, trichoid setae at apex of oviscapt..... 8
7. Female with distinct pale spots on 5-6Ts and small, faint pale spots on 4T; aedeagus laterally with serrations.....  
..... *dianensis* sp. nov.  
—Female with pale spots on 2T and 4-7Ts; aedeagus laterally without serrations.....  
..... *subsilvestris* Hardy & Kaneshiro
8. Cercus with setae nearly evenly distributed over; median lobe of decasternum broad..... *limingi* sp. nov.  
—Lower portion of cercus with more or less dense setae; median lobe of decasternum narrow ..... 9
9. Scutum with 2 obscure, longitudinal stripes (difficult to observe in dark specimens); apical tarsomeres darker than proximal ones; median lobe of decasternum long; anteroventral bridge of oviscapt long..... *bifasciata* Pomini  
—Scutum without longitudinal stripes; tarsomeres unicolor; median lobe of decasternum small, triangular; anteroventral bridge of oviscapt short.....  
..... *tsukubaensis* Takamori & Okada

# ACKNOWLEDGEMENTS

The authors wish to express sincere thanks to the following persons for invaluable comments on this manuscript and kind help in field collections and laboratory rearing: Prof. R.p. Kuang (South-west Forestry College, Kunming), Dr. H. Takamori (Tokyo Gakugei University), Dr. H.-w. Chen (Tokyo Metropolitan University), Dr. J. -g. Xiang-Yu (KIZ), Dr. L.-p. He (KIZ), and Mr. B. Duan (Yunnan Agricultural University). This work was supported by NSFC (39930100, 30024004), CAS (KSCX2-1-05), and JSPS (No.12375002).

# REFERENCES

- Bächli G, Rocha-Pité MTR (1981) *Drosophilidae* of the Palearctic region. In "The Genetics and Biology of *Drosophila*, vol 3a" Ed by Ashburner M, Carson HL, Thompson JN, Academic Press, London, pp 167–196
- Buzzati-Traverso AA, Scossiroli RE (1955) The *obscura* group of the genus *Drosophila*. *Adv Genet* 7: 47–92
- Cariou ML, Lachaise D, Tsacas L, Sourdis J, Krimbas C, Ashburner M (1988) New African species in the *Drosophila obscura* group: genetic variation, differentiation and evolution. *Heredity* 61: 73–84
- Dobzhansky T, Queal ML (1938) Genetics of natural populations, I. Chromosome variation in populations of *Drosophila pseudoobscura* inhabiting isolated mountain ranges. *Genetics* 23: 239–251
- Dobzhansky T, Powell JR (1975) *Drosophila pseudoobscura* and its American relatives, *Drosophila persimilis* and *Drosophila miranda*. In "Handbook of Genetics, vol 3" Ed by King RC, Plenum Press, New York, pp 537–587
- Lakovara S, Saura A (1982) The *obscura* group. In "The Genetics and Biology of *Drosophila*, vol 3b" Ed by Ashburner M, Carson HL, Thompson JN, Academic Press, London, pp 1–59
- O'Grady PM (1999) Reevaluation of phylogeny in the *Drosophila obscura* species group based on combined analysis of nucleotide sequences. *Mol Phylogenet Evol* 12: 124–139
- Okada T (1988) Taxonomic Outline of the Family *Drosophilidae* of Japan. In "Selected Papers by Dr. Toyohi Okada (1936–1988)" Ed by Suzuki K, The Association of the Memorial Issue of Dr. Toyohi Okada, Toyama, Japan, iii+412pp
- Powell JR, Dobzhansky T, Hook JE, Wistrand H (1976) Genetics of

- natural populations, XLIII. Further studies on rates of dispersal of *Drosophila pseudoobscura* and its relatives. *Genetics* 82: 493–506
- Sturtevant AH (1942) The classification of the genus *Drosophila* with the description of nine new species. *Univ Texas Publ* 4213: 5–51
- Takamori H, Okada T (1983) *Drosophila tsukubaensis*, a new species of the *obscura* group of the Genus *Drosophila* (Diptera, Drosophilidae) from Japan. *Kontyû Tokyo* 51: 265–268
- Throckmorton LH (1975) The phylogeny, ecology, and geography of *Drosophila*. In "Handbook of Genetics, vol 3" Ed by King RC, Plenum Press, New York, pp 421–469
- Udvardy, MDF (1969) Dynamic Zoogeography with Special Reference to Land Animals. Van Nostrand Reinhold, New York, 445pp
- Watabe H (1977) *Drosophila* Survey of Hokkaido, XXXIV. Seasonal variations of body color of *Drosophila testacea*. *J Fac Sci, Hokkaido Univ (VI)* 21: 21–30
- Watabe H, Toda MJ, Li G, Duan C, Imitty R, Entomack B, Muhtar A (1993) *Drosophilid fauna* (Diptera, Drosophilidae) of Chinese Central Asia. *Jpn J Ent* 61: 525–545
- Watabe H, Terasawa R, Lin FJ (1996) A new species of the *Drosophila obscura* species-group (Diptera, Drosophilidae) from China. *Jpn J Ent* 64: 489–495
- Watabe H, Sperlich D (1997) *Drosophila hubeiensis* (Diptera, Drosophilidae), a new species of the *Drosophila obscura* species-group from the mainland of China. *Jpn J Ent* 65: 621–633
- Watabe H, Bachmann L, Haring E, Sperlich D (1997) Molecular study on *Drosophila sinobscura* and *hubeiensis*, two sibling species of the *D. obscura* group. *J Zool Syst Evol Res* 35: 81–94
- Watabe H, Sidorenko, VS (1997) Three unrecorded species of drosophilid flies (Diptera, Drosophilidae) from Russia. *Far East Ent* 38: 8
- Wheeler MR (1981) The Drosophilidae: A taxonomic overview. In "The Genetics and Biology of *Drosophila*, vol 3a" Ed by Ashburner M, Carson HL, Thompson JN, Academic Press, London, pp 1–97

(Received February 12, 2003 / Accepted April 1, 2003)